Microbiological Transformation of Steroids

1-DEHYDROGENATION OF 17,21-DIHYDROXYPREGN-4-ENE-3,20-DIONE WITH *GLOMERELLA CINGULATA*

By LUIS COSTA PLA

Department of Biochemistry, Antibióticos, S.A., León, Spain

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The micro-organism *Glomerella cingulata* dehydrogenates 17,21-dihydroxypregn-4-ene-3,20-dione to 17,21-dihydroxypregna-1,4-diene-3,20-dione in high yield practically without by-products.

The formation of a double bond in the 1,2 position of the A ring of steroids by microbiological methods was first reported by Vischer & Wettstein (1953) and Fried *et al.* (1953). The microbiological dehydrogenation of 17,21-dihydroxypregn-4-ene-3,20-dione (I) (Reichstein's compound S) to 17,21-dihydroxypregna-1,4-diene-3,20-dione (II) (Scheme 1) by fungi of the genus *Fusarium* (*Fusarium solani*) and species of the genera *Calonectria*, *Ophiobolus* and *Alternaria* was reported by Vischer *et al.* (1955a). In a later paper the same authors describe how they obtained compound II by using fungi of the genus *Didymella* (*Didymella lycopersici* Kelb) (Vischer *et al.*, 1955b). Stoudt *et al.* (1955), produced the compound by using a culture of *Bacillus sphaericus*, Nobile *et al.* (1955) with *Corynebacterium simplex*, Shull & Kita (1955) with *Mycobacterium smegmatis* and Shull (1957) with species of *Protaminobacter*; Iizuka & Naito (1960) succeeded with some strains of *Pseudomonas* and Tsuda *et al.* (1961) with *Bacillus pulvifaciens*.

The present paper reports the microbiological dehydrogenation of 17,21-dihydroxypregn-4-ene-3,20-dione in the 1,2 position by a culture of the fungus *Glomerella cingulata*. Although 17,21-dihydroxypregna-1,4-diene-3,20-dione had already

been obtained by microbiological methods, this genus of micro-organisms normally introduces a hydroxyl group into steroid molecules (Tsuda *et al.*, 1958; Shirasaka & Tsuruta, 1961; Shirasaka *et al.*, 1960). In the literature there is no indication of the use of this fungus for C-1,2 dehydrogenation and this conversion is a further example of the influence of the culture media on the course of microbiological transformations of steroids. Other cases have been reported by Sutter *et al.* (1957) with *Mycobacterium lacticola* and *Corynebacterium simplex*.

As the i.r. triplet $1675\text{--}1666\text{cm}^{-1}$, $1631\text{--}1626\text{cm}^{-1}$, $1610\text{--}1605\text{cm}^{-1}$ is characteristic of the grouping $\Delta^{1,4}$ -3-keto (Jones & Dobriner, 1949; Jones & Herling, 1954; Peterson *et al.*, 1953) it was suspected that a dehydrogenation of Reichstein's compound S in the 1,2 position had taken place. Both the $[\alpha]_D^{25}$ of $+77^\circ$ and the contribution of -220° of the 1,2 double bond to the molecular optical rotation agreed with the values given in the literature. The presence of this double bond was confirmed by chromic oxidation to androsta-1,4-diene-3,17-dione and the identity of the incubation product was proved by comparison with an authentic sample of 17,21-dihydroxypregna-1,4-diene-3,20-dione.

A communication on this paper has been presented (Costa Pla, 1964).

Scheme 1.
For explanation see the text.

Experimental

Culture of the micro-organism

Starting from freeze-dried *Glomerella cingulata* A.T.C.C. 10534, slants with Saboureaud Difco medium were seeded and incubated for 7 days at 25°C. From three slants a spore suspension in sterile water (100ml) was prepared. Ten Erlenmeyer flasks (500ml) each containing 100ml of medium were seeded with 10ml of the spore suspension. The composition of the medium was as follows: 0.38% (w/v) Pharmamedia (Traders Protein Division, Fort Worth I, Texas, U.S.A.); 2.50% (w/v) glucose; 0.23% (w/v) corn-steep liquor. The pH value was 4.9 before sterilization and pH 5.9 after sterilization at 120°C for 20min. The flasks were incubated at 28°C for 48h in a rotary shaker (260 cycles/min).

Microbiological transformation

After the 48h preliminary incubation period, 17,21-dihydroxypregn-4-ene-3,20-dione dissolved in ethanol (100mg/4ml) was added to each flask. Incubation was continued at 25°C for 90h.

To monitor the transformation by paper chromatography, 2ml samples were taken at convenient times; these were extracted with 5ml of methylene chloride, then the solvent was distilled off and the residue dissolved in the necessary amount of acetone. The samples (about 100µg) were run on Whatman no. 1 paper alongside 17,21-dihydroxypregn-4-ene-3,20-dione in the system 1,2-propylene glycol (stationary phase)–toluene (mobile phase) for 48h at 25°C; ammoniacal AgNO₃ was used to locate the compounds (Neher, 1958).

These assays indicated the formation of a new steroid with a smaller *R_F* value than that of 17,21-dihydroxypregn-4-ene-3,20-dione and that after 90h the transformation was practically complete.

Extraction and characterization

After 90h the steroid in each flask was extracted twice with 200ml of methylene chloride. The solvent was evaporated and the residue dissolved in acetone; the solution was concentrated to a small volume and left to crystallize overnight at 0°C. The filtered crystals were washed with a little cold acetone and recrystallized to constant m.p., 230–234°C (decomp., Kofler, uncorrected). Yield 86%; found: C, 73.10; H, 8.37; C₂₁H₂₈O₄ requires C, 73.22; H, 8.19%. $[\alpha]_D^{25} + 77 \pm 2^\circ$ (c 0.8 in ethanol), molecular optical rotation ($[\alpha]_D^{25} \times \text{mol. wt.}/100$), +265°. The molecular optical rotation of 17,21-dihydroxypregn-4-ene-3,20-dione is +485° (Vischer *et al.*, 1955a) and the contribution of the 1,2-double bond to the molecular optical rotation is –220°. The product had i.r. spectrum bands (KBr) at: 3400cm^{–1} (OH); 2958cm^{–1} (CH); 1718cm^{–1} (CO); 1666cm^{–1}, 1620cm^{–1}, 1607cm^{–1} ($\Delta^{1,4}$ -3-one).

For the chromic oxidation to androsta-1,4-diene-

3,17-dione the method of Peterson *et al.* (1953) was used. The presence of androsta-1,4-diene-3,17-dione in the oxidation residue was confirmed by paper chromatography on Whatman no. 1 in the system: 1,2-propyleneglycol–acetone (1:4, v/v) as stationary phase and light petroleum (b.p. 86–100°C) as mobile phase. On the paper androsta-1,4-diene-3,17-dione (100µg), the oxidation residue (250µg) and the mixture of the two were spotted. After 5h of development at 25°C the steroids were detected with Zimmerman's reagent (Neher, 1958). The transformed steroid was identified as 17,21-dihydroxypregna-1,4-diene-3,20-dione by comparison with an authentic sample by mixed m.p., chromatographic mobility, u.v. and i.r. spectra.

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